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Immunomodulatory effect of *Schizonepeta tenuifolia* water extract on mouse Th1/Th2 cytokine production in-vivo and in-vitro

Hee Kang, Yoo-Joung Oh, Ho-Young Choi, In-Hye Ham, Hyun-Su Bae, Sung-Hoon Kim and Kyoo-Seok Ahn

Abstract

Schizonepeta tenuifolia (ST) is a major herbal constituent included in treatments for the common cold with fever, ostitis media and other skin inflammations. The present study investigated the effect of ST water extract on the pattern of cytokine production from activated T cells in-vivo and in-vitro. When ST (200 mg kg^{-1}) was given orally to mice for 7 days before i.v. injection of anti-CD3 antibody, it significantly decreased mRNA levels of interleukin (IL)-4, interferon (IFN)-γ and T-bet. Our flow cytometric analysis showed that ST administration significantly increased CD69 expression but showed little effect on the subsets of T cells. When we cultured mouse CD4 T cells under Th1/Th2 differentiation in the presence of ST, the suppressive activity of ST on IFN-γ involved T-bet, but the downregulation of IL-4 occurred independently of the Th2 transcription factors GATA binding protein 3 (GATA-3) and c-Maf. However, it increased IL-2 secretion during Th1/Th2 differentiation. Our study demonstrates that ST regulates inflammatory responses by reducing the release of Th1 and Th2 cytokines from T cells and prevents unprimed CD4 T cells from differentiating into Th1 and Th2 cells.

Introduction

CD4 T helper cells are divided into Th1 cells and Th2 cells, depending on the cytokine profile they produce in response to microbe or antigenic stimulation (Mosmann & Coffman 1989; Singh et al 1999; Glimcher & Murphy 2000; Rao & Avni 2000). The representative Th1 cytokine, interferon-γ (IFN-γ), contributes to cell-mediated and inflammatory immune responses, activating macrophages and thus eliminating intracellular bacteria and viruses, while Th2 cytokines such as interleukin (IL)-4, IL-5 and IL-13 are involved in humoral immunity, providing signals to B cells (Rengarajan et al 2000). However, such phenotypes can be influenced by a variety of factors such as the strength of T-cell receptor signalling, the nature of a pathogen or the type of antigen, the dose and route of the antigen and the genetic background of the host (Constant & Bottomly 1997; O'Garra 1998). Among these, the most decisive is the balance of surrounding cytokines at the beginning of contact with the microorganism or antigen. Therefore, transcriptions factors that determine the production of IFN-γ or IL-4, IL-5 and IL-13 are considered to be crucial in changing the relative ratio of Th1/Th2 cytokines (Kitamura et al 2005). T-bet, known as a key Th1 transcription factor, is upregulated in Th1 cells and induces IFN-γ production; on the other hand, GATA binding protein 3 (GATA 3) and c-Maf are the signature Th2 transcription factors. More specifically, GATA-3 induces all Th2 cytokine genes while c-Maf is identified as a transcription factor for the induction of IL-4 (Kim et al 1999).

The dried aerial parts of *Schizonepeta tenuifolia Briq*. are used in oriental medicine for the treatment of common cold, which includes symptoms such as fever, headache and sore throat, and skin rashes such as allergic dermatitis and psoriasis (Fung & Lau 2002). Clinically, *S. tenuifolia* (ST) is the major ingredient of several herbal formulas such as Hyungbangpaedoksan and Sopoongsan (Na et al 2006) for the applications described above. Studies on the pharmacological actions of ST demonstrate that it has anti-allergic effects by inhibiting mast-cell-mediated immediate-type hypersensitivity, and shows strong superoxide

Department of Pathology, College of Oriental Medicine, KyungHee University, Seoul, 130-710, South Korea

Hee Kang, Yoo-Joung Oh, Sung-Hoon Kim, Kyoo-Seok Ahn

Department of Herbology, College of Oriental Medicine, KyungHee University, Seoul, 130-701, South Korea

Ho-Young Choi, In-Hye Ham

Department of Physiology, College of Oriental Medicine, KyungHee University, Seoul, 130-701, South Korea

Hyun-Su Bae

Correspondence: Hee Kang, Department of Pathology, College of Oriental Medicine, Kyunghee University, 1 Hoegidong Dongdaemungu, Seoul 130-701, South Korea. E-mail: shehee@khu.ac.kr

scavenging activity (Shin et al 1999). In addition, ST is reported to have antipruritic, antimicrobial and haemostatic activities (Tohda et al 2000; Fung & Lau 2002).

In the present study, we report the immunomodulatory effects of ST water extract on the Th1/Th2 cytokine secretion pattern using an anti-CD3 injected mouse model in-vivo and mouse Th1/Th2 polarization conditions in-vitro.

Materials and Methods

Mice

Female BALB/c mice (8 weeks of age) were purchased from SCL Japan and maintained with rodent chow and water ad libitum in a temperature- and humidity-controlled pathogen-free animal facility at Kyunghee University. Mice were cared for according to the guidelines issued by the US National Institutes of Health (NIH publication 85-23, 1985) and the protocol was approved by our institutional ethical committee for animal welfare.

Reagents

RPMI, fetal bovine serum (FBS) and antibiotic–antimycotic were purchased from GIBCO-BRL. Recombinant interleukin (rIL)-4, rIL-12, anti-IL-4, anti-IL-12, anti-CD3e and anti-CD28 monoclonal antibodies (mAbs), and IL-4, IFN-γ and IL-2 OptEIA sets were from BD Pharmingen (San Diego, CA, USA). Recombinant IL-2 was purchased from Sigma (St Louis, MO, USA). Magnetic cell sorting CD4 (L3T4) microbeads were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). SuperScript Reverse Transcriptase was purchased from Invitrogen (Carlsbad, CA, USA). Power SYBR Green PCR Master Mix was purchased from Applied Biosystems (Foster City, CA, USA).

Preparation of ST water extract

S. tenuifolia plant material was purchased from Kyunghee University Medical Center Medicinal Herbs (Seoul, Korea), and its identification was authenticated by Professor Choi Ho-Young of the Department of Herbology, Kyunghee University. A voucher herbarium specimen (ST-2007) was deposited at the Department of Herbology. The plant sample was soaked in one volume of distilled water overnight, and further dissolved using sonication for 1 h. The extract was filtered and evaporated using a −70°C freeze dryer. The yield of the extract was about 2.67%. The sample was dissolved in phosphate-buffered saline (PBS) and sterilized by passing through a 0.22 μm syringe filter.

In-vivo T-cell activation

ST extract was suspended in PBS and given orally to mice at a dose of 200 mg kg^{-1} for 7 consecutive days. The dose is relevant to the therapeutic dose of oral ST. Normal and control mice were treated with the same amount of PBS. On day 8, mice were given a single i.v. dose of anti-CD3 Ab (40 μg mL^{-1} in PBS) (Scott et al 1990) and were killed 90 min later. The spleens were then removed and prepared for real-time RT-PCR and flow cytometry.

Splenocyte preparation and CD4 T cell isolation

Splenocytes were prepared by disrupting the spleen between glass slides in complete medium (RPMI 1640 with 10% FBS, 1% penicillin–streptomycin). After 10 min' centrifugation at 1000 rev min^{-1} to separate cells from debris, the cells were washed in RPMI medium, followed by lysis of erythrocytes. The cells were then counted, and viability was determined by trypan blue exclusion. CD4 T cells were isolated from splenocytes by positive selection using magnetic-activated cell sorter (MACS) CD4 (L3T4) microbeads, according to the manufacturer's instructions. In brief, CD4 T cells were separated by passing the cell suspension over a MACS MS column held in a MACS magnetic separator (Miltenyi Biotec). The CD4 T cells adhering to the column, yielding a purity of 97% confirmed by FACScan flowcytometry (Becton Dickinson, San Jose, CA, USA), were then used for further assays.

Measurement of cytokine and transcription factors by real-time RT-PCR

Total RNA from whole spleen cells or CD4 T cells was isolated using an RNeasy Mini Kit (Qiagen) and cDNA was reverse-transcribed by SuperScript Reverse Transcriptase. Oligonucleotide primers were designed using the Primer Express software (Applied Biosystems). The GAPDH gene was used as an endogenous control to normalize the expression of target genes. Quantitative real-time PCR was performed in triplicate using a 96-well optic tray on an ABI PRISM 7300 sequence detector (Applied Biosystems). Negative controls lacking template RNA were included in each experiment. The expression of each mRNA was quantified by relating the PCR threshold cycle obtained from the samples to amplicon-specific standard curves. All data are presented as mean ± s.d.

Proliferation assay

CD4 T cells were grown in 96-well plates coated with anti-CD3 Ab. Anti-CD28 Ab (2 μg mL^{-1}) were added to the cells, which were cultured in the presence of varying concentrations of ST for 24, 48, 72 h at 37°C in 5% CO_2. The proliferation capacity of CD4 T cells was determined using the CellTiter 96 non-radioactive cell proliferation assay (Promega). The assay is based on the cellular conversion of the tetrazolium salt MTS into a formazan product in the tissue culture medium, which can be measured spectrophotometrically at 490 nm.

In-vitro Th1 and Th2 cell polarization

CD4 T cells were activated with plate-bound anti-CD3e, 10 μg mL^{-1}, and anti-CD28, 2 μg mL^{-1}. For Th1 cell differentiation, CD4 T cells were incubated with 5 ng mL^{-1} rIL-12, 10 μg mL^{-1} anti-IL-4 Ab and 10 ng mL^{-1} rIL-2. For Th2 cell differentiation, 5 ng mL^{-1} rIL-4, 10 ng mL^{-1} rIL-2 and 10 μg mL^{-1} anti-IL-12 Ab were added to the culture medium. ST extract was added at the start of culture. After 3 days' culture, total RNA was isolated and supernatants were collected.

ELISA

Flat-bottomed 96-well plates were coated overnight at 4°C with coating mAbs. The primary mAbs were discarded and the plates were blocked with Assay Diluent (Pharmingen) for 1 h at room temperature. The plates were washed three times with wash buffer (0.05% Tween 20 in PBS) and blotted on a paper towel. Diluted samples or standards were added in triplicate. The plates were incubated for 2 h at room temperature. The supernatant was discarded and the wells were washed five times with wash buffer. Detecting mAb plus avidin–horse radish peroxidase was added and incubated for 1 h at room temperature. After washing, tetramethybenzidine substrate solution (Pharmingen) was added. The colour was allowed to develop for 30 min in the dark before the reaction was quenched with 0.2 M H_2SO_4. The plates were then read at 450–570 nm and the sample concentrations were determined from a standard curve.

Flow cytometry

Splenocytes (1×10^5) from control and ST-treated mice were stained with anti-CD3 FITC (CD3 ε chain) (145-2C11), anti-CD8a FITC (Ly-2) (53-6.7), anti-CD69 P-E (H1. 2F3), and anti-CD4 P-E (L3T4) (GK1.5) for 20 min on ice in the dark. After washing twice with PBS/0.1% NaN_3/1% FBS, the double-stained cells (live gated on the basis of forward and side scatter profiles) were analysed with a FACScan flowcytometer. The data were processed using Cell Quest software (Becton Dickinson).

HPLC analysis

ST extract (2 mg) was dissolved in 100 mL methanol (HPLC grade, Duksan Chemical, Korea) and ultrapure distilled water (resistivity > 18 Mω) and filtered through a 0.45 μm syringe filter (PVDF, Advantec, Japan). The standard material used for the quantitative analysis of ST was R-(+)-pulegone (Park et al 2006). The standard (1 mg) was dissolved to give serial concentrations (12.5, 25, 50, 100 μg mL^{-1}) and the standard HPLC chromatogram was obtained. The relationship between the concentration and the peak area was measured by the minimum square method (R^2 value). The HPLC apparatus was a Gilson system equipped with a 234 Autosampler, a UV/VIS-155 detector and a 321 HPLC pump (Gilson, Korea). A Luna 4.60 × 250 mm C_{18} reverse-phase column with 5 μm particles (Phenomenex, Torrance, CA, USA) was used. The mobile phase was acetonitrile (HPLC grade, Duksan Chemical) and water (with 0.01% formic acid) in the ratio of 50:50 (v/v), delivered at a flow rate of 1 mL min^{-1}. All solvents were degassed with a micromembrane filter (PTFE, Advantec). Ten microlitre volumes were analysed and the column eluent was monitored at UV 245 nm. Chromatography was performed at room temperature. The quantity of standard material of ST was as follows: the amount (mg) of standard materials = the quantitative amount (mg) of standard materials × AT/AS/n (n = 3; AT = the peak-area of the test sample containing standard materials; AS = the peak-area of standard material).

Statistical analysis

Statistical differences among the means of multiple groups were determined by one-way analysis of variance followed by Dunnet's post-hoc test. The difference between the two means was assessed using a non-paired Student's *t*-test. Calculations were carried out using SPSS version 12. *P* values less than 0.05 were considered significant.

Results

Effect of ST extract on transcription of IFN-γ, IL-4 and Th1/Th2-specific transcription factors in anti-CD3 antibody injected mice

Initially, we evaluated the in-vivo effect of short-term oral administration of ST on Th1/Th2 cytokines and their specific transcription factors T-bet, GATA-3 and c-Maf. A single i.v. injection of anti-CD3 antibody can rapidly induce high levels of Th1 and Th2 cytokine mRNAs. At 90 min after anti-CD3 stimulation, control and ST-treated mice showed significantly higher levels of mRNA than normal mice (Figures 1A, B).

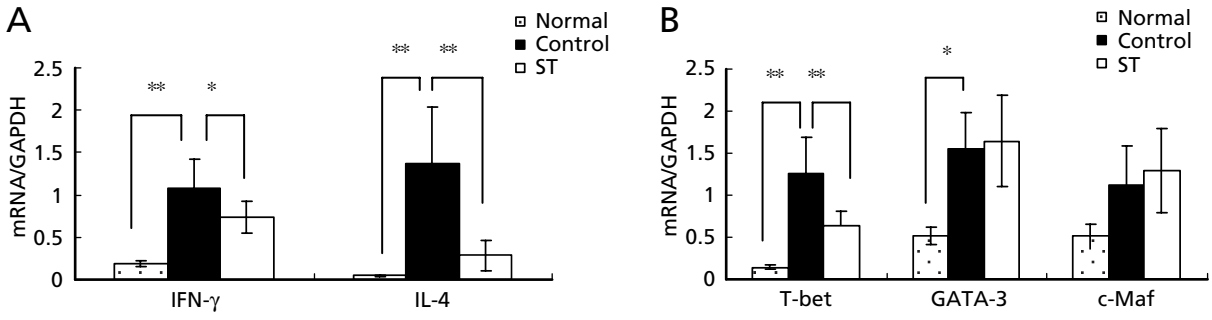

Figure 1 Real-time RT-PCR analysis of target gene expression (IFN-γ and IL-4 (A) and T-bet, GATA-3 and c-Maf (B)) in splenocytes from mice injected with anti-CD3 antibody (Ab). *Schizonepeta tenuifolia* (ST) extract (200 mg kg^{-1}) was given orally to Balb/c mice for 7 consecutive days. On day 8, mice were given an i.v. injection of anti-CD3 Ab. The spleen was removed 90 min after this injection and the total RNA extracted and real-time RT-PCR performed. Transcripts of interferon (IFN)-γ and interleukin (IL)-4 (A), T-bet, GATA-3 and c-Maf (B) were normalized to GAPDH expression. Data are mean ± s.d. of three independent assays. *$P < 0.05$; **$P < 0.005$ (analysis of variance followed by Dunnet's post-hoc test).

ST treatment significantly downregulated transcription of IFN-γ and IL-4 by 37% ($P<0.05$) and 80% ($P<0.001$), respectively, compared with control mice (Figure 1A). With regard to Th1/Th2-specific transcription factors, T-bet expression was significantly reduced by 50% by ST treatment ($P<0.005$) but expression of GATA-3 and c-Maf was little affected (Figure 1B). Our findings suggest that for the Th2 cytokine response, ST may interfere with other pathways in IL-4 signalling that do not involve GATA-3 or c-Maf, while it can inhibit at least one IFN-γ signalling pathway at the transcription level.

Effect of ST extract on splenocyte subsets in anti-CD3 antibody injected mice

We examined whether oral treatment with ST might influence any quantitative and qualitative changes in the spleen-derived T-cell populations. As shown in Table 1, anti-CD3 injection did not change the subsets of T cells but significantly induced CD69 expression ($P<0.001$), an early activation marker (Ziegler et al 1994). ST treatment significantly increased CD69 expression on T cells ($P<0.05$) but showed little effect on the percentage of CD4 or CD8 T cells. Unlike the downregulated transcription of cytokines described above, our flow cytometry data suggest that ST did not suppress the activity of T cells.

Effect of ST water extract on CD4 T-cell proliferation in-vitro

We isolated CD4 T cells from mouse spleen and evaluated the proliferation of CD4 T cells activated with anti-CD3 plus anti-CD28 in the presence of ST extract. In our previous test, we confirmed that the ST extract alone did not induce T-cell activation, as measured by CD69 expression, nor did it induce mitogenic responses, assessed using the MTS assay (data not shown). As shown in Figure 2, ST had minimal effects on the proliferation of activated CD4 T cells at 24 h and 48 h but treatment for 72 h at a dose of 100 μg mL^{-1} showed a 20% reduction compared with untreated cells.

Table 1 Effects of *Schizonepeta tenuifolia* (ST) extract on splenocyte subsets in mice injected with anti-CD3 antibody (Ab). ST extract was given orally to Balb/c mice for 7 consecutive days; on day 8, mice were given an i.v. injection of anti-CD3 Ab. Splenocytes were obtained 90 min after the injection and analysed for CD4, CD8 and CD69

	Normal	Control	ST
CD4(+) CD3(+)	28.88 ± 3.67	28.51 ± 2.12	32.57 ± 2.91
CD8(+) CD3(+)	9.26 ± 0.53	9.04 ± 0.97	8.98 ± 0.81
CD69(+) CD3(+)	12.18 ± 1.36	80.35 ± 1.55*	83.41 ± 2.39†

Data are mean percentage of cells ± s.d. in three independent assays (n = 5). *$P<0.001$ vs normal; $^{\dagger}P<0.05$ vs control (analysis of variance followed by Dunnet's post-hoc test).

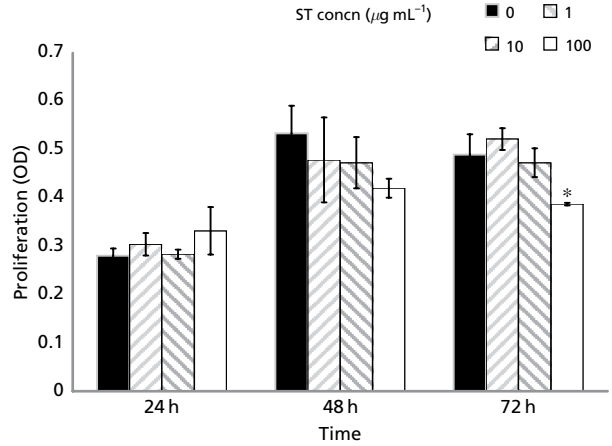

Figure 2 Time- and dose-dependent effects of *Schizonepeta tenuifolia* (ST) extract on CD4 T cell proliferation in response to anti-CD3 antibody (Ab)/anti-CD28 Ab. CD4 T cells from BALB/c mice were sorted and stimulated with anti-CD3/anti-CD28 in the presence of varying concentrations (1, 10 and 100 μg mL^{-1}) of ST for 48 h. Proliferation was quantified using the MTS assay. Results (optical densities) were calculated as the percentage of control cultures. Data are mean ± s.d. of three independent assays. *$P<0.05$ vs control culture (non-paired Student's t-test).

Effect of ST water extract on cytokine production in Th1/Th2 differentiation in mouse CD4 T cells

We determined whether the ST extract could influence the differentiation of CD4 T cells under Th1 or Th2 polarization conditions in-vitro. Table 2 shows that ST significantly inhibited the secretions of IFN-γ and IL-4 from Th1 and Th2 cells in a dose-dependent manner. Given the proliferation data described above (Figure 2), the decrease in IFN-γ and IL-4 production cannot be solely accounted for by the change in cell number. In addition, the levels of IL-2, which is critical for the growth of T cells, were significantly increased in both Th1 and Th2 cells. These data show that ST treatment is able to suppress the populations of committed Th1 and Th2 cells.

Effect of ST water extract on transcription of Th1/Th2 cytokines and their specific transcription factors in Th1/Th2 differentiation in-vitro

Our real-time RT PCR analysis (Figure 3) showed that the ST water extract significantly decreased the transcription of IFN-γ and T-bet in a dose-dependent manner: by 53% and 62%, respectively, at 10 μg mL^{-1}, and by 90% and 77%, respectively, at 100 μg mL^{-1}, which is in parallel with the in-vivo data. As for IL-4, a 36% increase was observed at 10 μg mL^{-1} ($P<0.05$), but its expression was reduced by 28% at 100 μg mL^{-1} ($P<0.05$). The inhibitory effects of ST on the expression of GATA-3 and c-Maf were minimal. On the whole, the inhibitory effect of ST was more effective with IFN-γ than with IL-4 because of the synergistic downregulation of the transcription of T-bet and IFN-γ.

Table 2 Effects of *Schizonepeta tenuifolia* extract (ST) on cytokine production during mouse Th1/Th2 cell differentiation in-vitro. CD4 T cells were incubated with recombinant interleukin (rIL)-2, rIL-12 and anti-IL-4 antibody (Ab) for Th1 differentiation or with rIL-2, rIL-4 and anti-IL-12 for Th2 differentiation for 72 h. Levels of interferon (IFN)-γ, IL-4 and IL-2 were determined from supernatants using ELISA

	ST ($\mu g mL^{-1}$)	IFN-γ ($ng mL^{-1}$)	IL-4 ($ng mL^{-1}$)	IL-2 ($ng mL^{-1)}$
Th1 polarization	0	432.33 ± 15.30	–	81.60 ± 0.52
	10	203.15 ± 37.00*	–	99.94 ± 6.91*
	100	110.32 ± 8.89*	–	163.71 ± 3.60*
Th2 polarization	0	–	19.01 ± 0.06	55.67 ± 3.38
	10	–	15.69 ± 0.20*	64.71 ± 1.87*
	100	–	7.38 ± 0.34*	127.46 ± 6.91*

Data are mean ± s.d. of three separate experiments. *$P < 0.05$ vs control culture (non-paired Student's *t*-test).

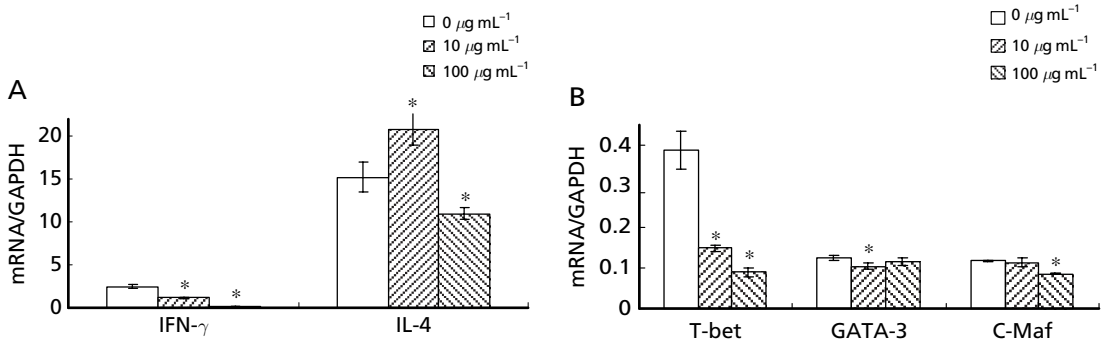

Figure 3 Effects of *Schizonepeta tenuifolia* (ST) extract on interferon (IFN)-γ, T-bet, interleukin (IL)-4, GATA-3 and c-Maf gene expression during mouse Th1/Th2 cell differentiation in-vitro. Th1/Th2 cell differentiation was carried out as described in Table 2. Total RNA was isolated on day 3 and transcriptional levels of IFN-γ and IL-4 (A), T-bet, GATA-3 and c-Maf (B) were analysed by quantitative real-time RT-PCR. Data are the mean ± s.d. of three independent experiments. *$P < 0.05$.

Quantifiation of pulegone in ST water extract

To identify pulegone as the active constituent of ST (Fung & Lau 2002), we performed HPLC. The standard curve was calibrated by using the linear regression derived from the peak area (Figure 4). The regression equation (correlation coefficient, R^2) of pulegone was $y = 24535x - 21260$ (0.9998), which exhibited good linearity. The content of pulegone in 1 g ST extract was 48.65 mg, which corresponds to 0.29% of the whole extract.

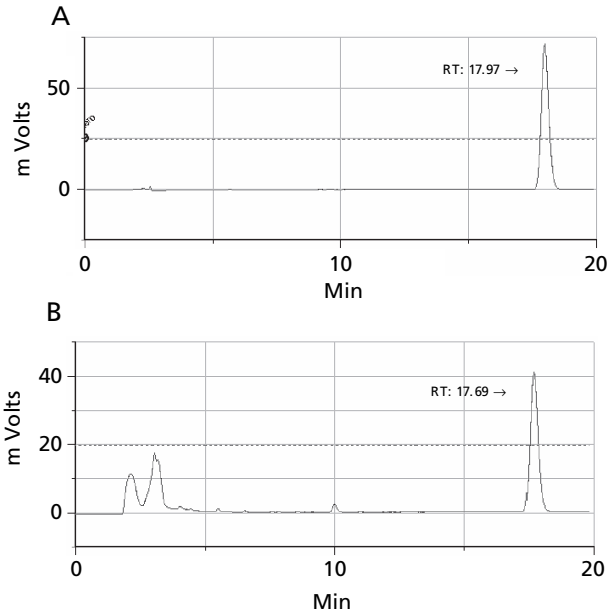

Figure 4 HPLC chromatograms of ST pulegone: (A) standard sample; (B) pulegone in ST.

Discussion

In this study, we demonstrated that the water extract of ST suppresses the transcription of IFN-γ and IL-4 when T cells are activated in-vivo and that it also inhibits the production and transcription of IFN-γ and IL-4 under the Th1/Th2 differentiation culture in-vitro.

More and more traditional herbs are favoured in clinical settings as they are believed to cause little adverse effect on the individual immune status. Data on the immunological aspects of traditional herbal medicines are therefore needed, as a shortage of scientific evidence is often a stumbling block to the growing use of herbs. ST is one of the most frequently

used herbal ingredients for psoriasis and allergic eczema. Psoriasis is characterized by over-production of IFN-γ (Uyemura et al 1993), while allergic eczema is explained by hypersensitivity of immunoglobulin E with the help of IL-4 (Pène et al 1988); this led to us to examine the immunomodulatory effect of ST extract on mouse T-cell cytokines.

Since ST is applied in allergic skin rashes, the possibility that ST might be antigenic or mitogenic was raised; our preliminary test confirmed that it did not induce mitogenic or antigenic property (data not shown). Instead, we wanted to examine how ST can modulate activated T cells. When mice were stimulated with i.v. anti-CD3 antibody after 1 week of oral administration of ST extract, a minor but still significant increase in CD69 expression was observed, and there was a significant reduction in the transcription of IFN-γ and IL-4 in the spleen. Our further evaluations of Th1/Th2-specific transcription factors showed that oral administration of ST significantly affected T-bet, but not GATA-3 or c-Maf. Such effects occurred in the differentiation of Th1/Th2 cells in-vitro in which IFN-γ and IL-4 were downregulated at both the protein and mRNA levels. T-bet was significantly inhibited whereas a slight decrease in c-Maf was observed at a high dose. GATA-3 and c-Maf upregulate IL-4 transcription by binding to either the IL-4 promoter or the IL-4 enhancer (Kim et al 1999; Avni et al 2002). STAT-6 and NFAT1 are also involved in IL-4 expression: they not only bind to the promoter and enhancer of IL-4 but also enhance the induction of GATA-3 or c-Maf (Agarwal et al 2000). We postulate that ST may affect IL-4 expression by interfering with the ability of NFAT1 or STAT6 to bind to the IL-4 promoter or the interaction between STAT6 and GATA-3. In particular, NFAT1 is non-selective in binding to the promoter of IL-4 or that of IFN-γ in Th1 and Th2 cells (Szabo et al 1997; Kuo & Leiden 1999), and it is possible that ST may interfere with the activity of NFAT1 in Th1 and Th2 cells. Further studies are required to examine the effect of ST on STAT proteins and NFAT1 at a nuclear level.

Interestingly, ST treatment increased IL-2 levels in the supernatant from Th1/Th2 polarizing cultures. In fact, our in-vivo serum data also showed increased IL-2 levels in ST-treated mice (data now shown). During Th1/Th2 differentiation, a group of CD4 T cells, called Thpp cells, which are uncommitted and produce IL-2 but not IFN-γ or IL-4, prevent bipolar T helper precursor cells from differentiating into Th1 and Th2 cells (Akai & Mosmann 1999; Wang & Mosmann 2001). However, these Thpp cells, once appropriately restimulated, can differentiate into Th1 or Th2 cells. We can therefore assume that enhanced IL-2 production by ST partly influenced the outcome of the Th1/Th2 cytokine synthesis. This has to be confirmed by an in-vivo polarization model, such as experimental allergic inflammation or autoimmune disease.

Conclusions

Taken together, our results provide pharmacological evidence that ST water extract significantly downregulates IFN-γ and IL-4, the major T cell signature cytokines, in activated mouse T cells in-vivo. The downregulation of both cytokines by ST was also observed when mouse CD4 T cells were cultured under Th1/Th2 differentiation cultures. The suppressive activity of ST on IFN-γ involved the transcription of T-bet, but the downregulation of IL-4 occurred independently of GATA-3 and c-Maf. However, ST was able to increase IL-2 secretion from differentiating Th1/Th2 cells, suggesting that it can prevent unprimed CD4 T cells from differentiating into Th1 and Th2 cells. In conclusion, our study demonstrates that ST downregulates inflammatory responses by reducing the release of Th1 and Th2 cytokines from T cells and thus can be used for diseases that are associated with an imbalance of Th1/Th2 cytokines.

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